

Effect of Nystatin with and without DMAHDM on Antifungal and Mechanical Properties of Acrylic Resins

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ABSTRACT

Purpose: To investigate antifungal and mechanical properties after the impregnation of Dimethyl Amino-ethyl Hexa-decyl Di-methacrylate (DMAHDM) alone or in combination with Nystatin in polymethylmethacrylate. *Methodology:* The control group was fabricated by mixing powder and liquid of PMMA at the ratio of 2.5:1g/mL. The DMAHDM was added to PMMA liquid and were mixed with PMMA powder. The Nystatin (500,000 International Units (IU)) was mixed with PMMA powder, whereby the composite powder was mixed with the DMAHDM-based liquid. The prepared specimens were tested for fungal adhesion testing (at days 1 and 30), impact strength and flexural strength. *Oneway ANOVA post-hoc Tukey's test* were used for statistical analysis. *Results:* Statistical analysis for the adhesion assay revealed that the antifungal activities of unaged and aged specimens in experimental groups were statistically significant as compared to control group A. The groups containing DMAHDM with Nystatin have shown statistically reduced flexural strength. The impact strength test revealed that groups containing 20% DMAHDM alone and DMAHDM with Nystatin showed statistically reduced impact strength compared to the control group. *Conclusion:* Antifungal activities of experimental PMMA resin was increased. The addition of DMAHDM alone in PMMA resin has no deleterious effects on impact and flexural strength, however, at higher concentration values were reduced.

INTRODUCTION

Treatment of edentulism with implant-retained prosthesis is the ideal solution; however, being expansive, its use is limited. Polymethyl methacrylate (PMMA) is alternatively the material of choice and has been widely used to restore edentulism.¹ Despite the numerous advantages of PMMA resin as a dental polymer, it is not considered an ideal denture base material because of relatively low viscoelastic behavior to withstand high occlusal stresses in long run, insufficient mechanical strength to withstand impacts caused by sudden drop, and more susceptibility to microbial adhesion (*Candida albicans*) due to its weak surface properties, especially excessive surface roughness.² The removable denture itself is believed to serve as both a trauma inducer and a reservoir for triggering a local microbial infection-mediated inflammatory response.³

Denture-related stomatitis (DRS) with a prevalence of 15-70%⁴ is an inflammatory condition of the mucosa underneath the dentures triggered by microorganisms.^{5,6} *Candida albicans* have been established as a primary

etiologic agent, whereby denture stomatitis is not solely a result of *Candida albicans*. Instead, it is an outcome of multi-species biofilms that may include *Streptococcus mutans*, *Staphylococcus aureus*, *Candida glabrata*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida krusei*, and *Candida tropicalis* being the most commonly described. Mixed *C. albicans*, and *C. glabrata* biofilms may play an essential role in the pathogenesis associated with severe inflammation in denture wearers.⁷

The switch between yeast and mycelial growth is one of the virulence factors, which can also lead to *Candida* biofilm formation.⁸ Reducing *Candida* biofilm on the denture surface is reasonable to control denture stomatitis. Although adequate denture cleaning is vital for its prevention, it is more advantageous and crucial to developing denture surfaces with antifungal properties.⁹ Several studies have been conducted to introduce antimicrobial polymers.^{10,11} These agents can be divided into agent releasing and non-agent releasing antimicrobials materials.¹¹

The literature shows that modifying resins by adding soluble antimicrobials such as antibiotics, fluoride, silver, tea tree oil, and a chlorhexidine.^{12,13} It is known that the antibacterial activity of such modified resins decreases with time, whereby mechanical or physical properties of the parent resin may be compromised by the constant release of such antibacterial agents. The porous surface formed during the release process leads to poor wear resistance, staining, and bacterial biofilm accumulation.¹⁰ To overcome this issue, researchers have developed non-volatile polymerizable Quaternary Ammonium Salts (QAS) that carries antimicrobial potency, e.g., 12-Methacryloyloxy dodecylpyridiniumbromide (MDPB) and Methacryloyloxyethylcetyl dimethyl ammonium chloride (DMAE-CB), chemically immobilized within resins.^{14,15} Their incorporation into resin materials is limited during polymerization because there is only one double bond in their molecular structures. 2-methacryloyloxyethyl hexadecyl methyl ammonium bromide resin (MAE-HB) has two double bonds with strong and long-lasting antibacterial effects.^{10,16}

Previous studies demonstrated the antifungal action of different QAS, i.e., 2-methacryloyloxyethyl phosphorylcholine (MPC)/Dimethylaminohexadecyl methacrylate (DMAHDM) and dimethylamino dodecyl methacrylate (DMADDM) along with chlorhexidine in denture lining, acrylic resins, resin-composites, and adhesives systems.^{17,18} When added into a resin composite, a quaternary ammonium dimethacrylate (QADM) has exhibited excellent anti-biofilm properties.¹⁹ It has also been proved that the antimicrobial property of Quaternary Ammonium Monomers (QAMs) increased as the alkyl chain length increased from 5 to 16 and deteriorated when chain length was further increased to 18. Therefore, Dimethylaminohexadecyl methacrylate (DMAHDM), having a chain length of 16, showed the strongest antibacterial activity comparatively.²⁰

Denture fractures are particularly common and cause issues for patients, dentists, and laboratory staff. Flexural fatigue and impact force are the two main causes of denture fractures.

Midline fracture is caused by flexural fatigue, while denture fracture from abrupt denture fall is caused by impact failure.²¹ Denture fracture can be caused by a variety of causes, including a thin denture base, a prominent frenum-usually labial, a prominent mid-palatine raphe, and a single complete denture opposing natural dentition without any reinforcement.²² The most common site of denture fracture is the midline (59%), which usually coincides with the notch for labial frenum relief on either the upper or lower complete denture. Thus, denture fracture demonstrates that sufficient flexural and impact strength are frequently required to resist these fractures.²³

No study has been conducted in previous literature on incorporation of conventional PMMA with DMAHDM and Nystatin in combination for their fungicidal effects and mechanical properties. The rationale of this study is to evaluate the performance of denture base material after adding the anti-fungal agents.

The aim of this study is to investigate the effects of DMAHDM and Nystatin agents on the fungicidal effects and mechanical properties of PMMA denture base resin. To improve the effectiveness of denture base resin and reduce the colonization of *Candida*, the impregnation of DMAHDM and Nystatin agents into the resin has been proposed. Using dual antimicrobial agents may be a reasonable therapy in the treatment of denture related stomatitis by interrupting the cycle of reinfection without affecting the mechanical properties.

MATERIALS AND METHODS

The study was approved by the Institutional review board of the Prime Foundation Pakistan, Riphah International University, Islamabad, Pakistan. Heat-cured Polymethyl methacrylate acrylic resin polymer powder and liquid (MEADWAY, Bracon, UK), 2-(dimethylamino) ethyl methacrylate liquid (Sigma-Aldrich, Taufkirchen, Germany), 1-bromohexadecane liquid (Sigma-Aldrich, Taufkirchen, Germany), Nystatin powder (Vltavska 53, 252 63 Rostoky, Czech Republic), and Ethanol Absolute (Merck KGaA, 64271 Darmstadt, Germany) were used in this study. DMAHDM was synthesized via a modified Menschutkin reaction method as reported previously.^{20,24}

For the control group, powder and liquid of PMMA heat cure resin were taken in a ratio of 2.5:1 according to standard dental laboratory usage as described previously.²⁵ For DMAHDM containing experimental groups, the DMAHDM was mixed to heat-polymerizable liquid, blending to a mass fraction of 5%, 10%, and 20%, which was 0.42 mL, 0.84 mL, and 1.68 mL, respectively of DMADDM into 7.98 mL, 7.56 mL, and 6.72 mL of monomers respectively. For Nystatin containing experimental groups, 20.89 g of PMMA powder and 102.99 mg (500000 IU) Nystatin were mixed by a custom-made ball milling machine at 1200 rpm for 10 min. The description and distribution of groups are tabulated in Table 1.

Table 1. Concentrations of DMAHDM and Nystatin in various groups.

S. no	Group	Concentrations
1.	A	Conventional Heat Cure Acrylic resin (as supplied)
2.	B1	Heat Cure Acrylic Resin modified with 5%. DMAHDM
3.	C1	Heat Cure Acrylic Resin modified with 5%. DMAHDM and Nystatin 500000 units
4.	B2	Heat Cure Acrylic Resin modified with 10% DMAHDM
5.	C2	Heat Cure Acrylic Resin modified with 10% DMAHDM and Nystatin 500000 units
6.	B3	Heat Cure Acrylic Resin modified with 20% DMAHDM
7.	C3	Heat Cure Acrylic Resin modified with 20% DMAHDM and Nystatin 500000 units

The prepared heat-cured resins for the control and experimental groups at the dough stage were packed in specific dies. Stainless steel alloy was used for die fabrication and was prepared by a CAD-CAM milling machine (CNC350; Arix Co. Tainan Hsin, Taiwan) for the flexural strength, impact strength, and fungal adhesion tests. The dimensions of the die for flexural strength, impact test, and microbiology tests were 65 mm × 10 mm × 3 mm, 80 mm × 10 mm × 4 mm, the width under notch bn was 9.75 mm, and 10 mm × 10 mm × 2 mm of length, width, and height, respectively. The mold was cleaned with a cotton cloth before each wax pattern fabrication so that the dimensional integrity of the wax pattern was kept intact. Modelling wax was used to make patterns using the mold to prepare the specimen. Each of the set wax patterns was then removed from their respective die. Those wax patterns having the devised dimensions were then invested in curing flask using standard laboratory steps. The internal surfaces of the flask compartments were cleared, dried, and smeared with Vaseline. The wax patterns were invested in each flask using dental stone (type III). The stone mix was mechanically vibrated to avoid air voids entrapment. The flask compartments were pressed (9.8 MPa) into the hydraulic press for 5 min before clamping²⁶ and then placed into a water curing bath (Acrydig 12, Italy) at room temperature and raising the temperature gradually to reach 100°C and maintained the temperature for half an hour. After completing the polymerization cycle, the flasks were allowed to bench cool overnight before opening. After opening, the specimens were recovered from flasks by the model cutter and immersed in distilled water at 37°C for 24 h for residual monomer elimination.

The surplus resin was carefully and gently removed by tungsten carbide bur using a marathon SDE – M35LS slow speed acrylic trimming headpiece. The specimens were finished with

200, 300, and 600 grit sandpapers and polished using a wet rag wheel (Botiflex, Germany) with pumice suspension. The same procedure was repeated for all specimens of the control group and experimental groups. A total of 280 samples were fabricated, free from any imperfection, including visible porosities, cracks, fractures, and bends.

FUNGAL ADHESION TEST

Candida albicans species was used for testing the antimicrobial activity for all specimens. Selected specimens in all groups were disinfected (6% NaOCl and 70% ethanol) before exposure to antifungal tests. The *candidal* suspension was prepared from 48 h cultures in Sabouraud Dextrose Broth (SDB). This suspension was standardized to 1×10⁸ CFU/mL with sterile normal saline to achieve the turbidity of 0.5 McFarland. Seventy disinfected and cleaned acrylic resin specimens of both control and experimental groups were immersed in this suspension. These samples were incubated for one hour at 37°C and then washed with sterile normal saline for 5 s to remove planktonic fungal cells. After washing, acrylic specimens were tapped and placed into separate test tubes having 1 mL sterile normal saline. Each sample was spun at 3000 rpm for 20 s to remove adhered fungal cells. Three folds (ten, hundred and thousand folds) serial dilutions of the suspension were done. The diluted suspension was inoculated on Sabouraud dextrose agar (SDA) petri dishes with sterile cotton swabs. These Petri dishes were incubated for 48 h at 37°C, and colonies of *C. albicans* were quantified by using the colony count method (marker mark method).

In the second phase of antifungal testing, 70 resin specimens (n=10) from control and experimental groups for antimicrobial tests were submerged and stored in distilled water in a sealed polyethylene container for 30 days at 37°C. After 30 days, the specimens were disinfected before subjecting to microbial tests. Adherence assay was performed after aging using the same procedure for unaged samples.

FLEXURAL STRENGTH TESTING

ISO 20795-1:2013²⁷ standard was used to measure the flexural strength of the 70 prepared specimens (n=10) with a universal testing machine using a three-point bending test (EZ20, Loyld Instruments Ltd., West Sussex, UK). The samples were stored in distilled water at 37°C for 24 h before testing for flexural strength. Immediately after removal from water, the samples were placed on the universal testing machine. The distance between the specimen supports was 50 mm. The loading force was applied to the specimen at a 5 mm/min crosshead speed until the sample fractured. The following formula was used to find the flexural strength. Additionally, the maximum load exerted on the specimens was recorded.

$$F.s = 3WL/2bd^2 \quad (\text{eq. 1})$$

Where flexural strength (F.s) is calculated in MPa, L is the space between supporting ends of the crosshead (50 mm); 7.5 mm on the outer side of supporting ends on each side, b: specimen width (10 mm); d: specimen thickness (3 mm); W: load recorded at fracture.

IMPACT STRENGTH TESTING

Charpy V-notch impact test was carried out using pendulum impact tester to record the Impact strength as outlined in ISO 179-1:2000.²⁸ The prepared resin specimens of all groups were kept at 37°C for 24 h before the impact strength test. The dimensions of the resin specimens were 80 mm × 10 mm × 4 mm with the V-notch length 0.25±0.05 mm at the center with angle notch sensitivity (rn) 45°±1° and span support 62±0.5 mm. The impact strength was calculated as follows;

$$IS = E/b_n d \times 10^3 \quad (\text{eq. 2})$$

Where, E: the value of energy absorbed by the specimen, where the impact resistance (J); b_n : specimen width and d: specimen thickness.

STATISTICAL ANALYSIS

Data analysis was done using SPSS (IBM Software, Armonk, NY, USA) version 2016. Statistical analysis was done using one-way analysis of variance, and the inter-comparison between each group was done using Post-hoc Tukey (HSD) tests. A p-value of < 0.05 was considered statistically significant.

RESULTS

FUNGAL ADHESION TEST

Figure 1 (a and b) showed that adding DMAHDM and Nystatin to PMMA acrylic resin reduced the candida adhesion on unaged and aged samples. The one-way ANOVA and post-hoc Tukey's outputs revealed that the antifungal activities of unaged aged samples in the experimental groups B1, B2, B3, C1, C2, and C3 were 0.4×10^3 CFU/mL, 0.3×10^3 CFU/mL, 0.4×10^3 CFU/mL, 0.8×10^3 CFU/mL, 0.3×10^3 CFU/mL, 0.2×10^3 CFU/mL respectively had shown results statistically significant ($p = 0.000$) compared to the control group A (4.8×10^3). The one-way ANOVA and post-hoc Tukey's outputs revealed that the antifungal activities of 30 days aged samples in the experimental groups B1, B2, B3, C1, C2, and C3 were 2.2×10^3 CFU/mL, 1.1×10^3 CFU/mL, 1.1×10^3 CFU/mL, 0.4×10^3 CFU/mL, 0.6×10^3 CFU/mL, and 0 respectively showing statistical significance ($p = 0.000$) compared to the control group A.

The highest antifungal activities were observed in the unaged and aged specimens in group C3 (0.2×10^3 and 0 CFU/mL) containing DMAHDM 20% and Nystatin. The control group had the lowest antifungal activities among the other groups. However, post-hoc Tukey's revealed that the antifungal activity of group B1 was not significant statistically compared to B2, B3, C1, C2 and C3 ($p > 0.05$). The results of group B2 compared

to groups B3, C1, C2, C3, and C1 compared to groups C2 and C3 were not significant statistically ($p > 0.05$). Similarly, there was no difference statistically in antifungal activities of groups C2 and C3 ($p > 0.05$).

MECHANICAL TESTING

The mean and standard deviation values of impact and flexural strength are given in Figures 2 and 3, respectively. One-way ANOVA revealed a statistical significance difference between and within the groups for both tests, i.e., impact strength and flexural strength ($p < 0.05$). Post-hoc Tukey's test revealed that the results of impact strength of groups B3, C1, C2, and C3 (2.19 ± 0.4 MPa, 2.26 ± 0.3 MPa, 1.96 ± 0.2 MPa and 1.78 ± 0.3 MPa) decreased significantly as compared to control group A (3.53 ± 0.65 MPa) having p value = 0.000. However, the experimental group B1 and B2 having impact strength values of 3.57 ± 0.5 MPa and 3.18 ± 1.08 MPa respectively showed no significant difference ($p = 0.707$). Whereas experimental groups B1 and B2 compared to groups B3, C1, C2, and C3 had shown statistically increased value ($p = 0.000$). The impact strength of Group C1 had shown statistically decreased values compared to group B3 with a p-value of 1.000. The groups B3 and C1 compared to C2 and C3 have shown no statistical difference.

Post-hoc Tukey's test revealed that the experimental groups C1, C2, and C3 having mean values 82.91 ± 6.98 MPa, 83.44 ± 6.15 MPa and 74.92 ± 4.8 MPa respectively had shown significantly decreased flexural strength compared to control group A (94.75 ± 7.78 MPa), showing a p-value of 0.003, 0.006, and 0.000. However, experimental groups B1, B2, and B3 having mean values 103.34 ± 7.81 MPa, 88.83 ± 6.64 MPa and 87.88 ± 6.01 MPa were not statistically significant compared to control group A having a p-value of 0.076, 0.434, and 0.259, respectively. The B1 group was significantly high compared to group B2, B3, C1, C2, C3 and group B2 was statistically high compared to group C3. Whereas, groups B3 and C1 were statistically non-significant as compared to group C3.

DISCUSSION

A variety of microorganism takes advantage of the environment generated by dentures and use them as a substrate for colonization.²⁹ Although PMMA has been widely used as denture base material, it has limitations due to its lack of antimicrobial activity.^{25,30} It is known that fungal infections caused by *C. albicans* related to denture base colonization are becoming a serious clinical problem in dentistry. Denture base PMMA resin with antimicrobial properties would be ideal for improving the patients' quality of life and impeding discomfort with cost-effectiveness. In order to overcome this negative behavior in denture base PMMA resin, the DMAHDM at a concentration of 5%, 10%, and 20% was incorporated according to Chen., et al. 2017²⁴ alone and with Nystatin were impregnated to heat-cured acrylic resin in the current study to have a synergistic effect on the antifungal activities against *C. albicans*.

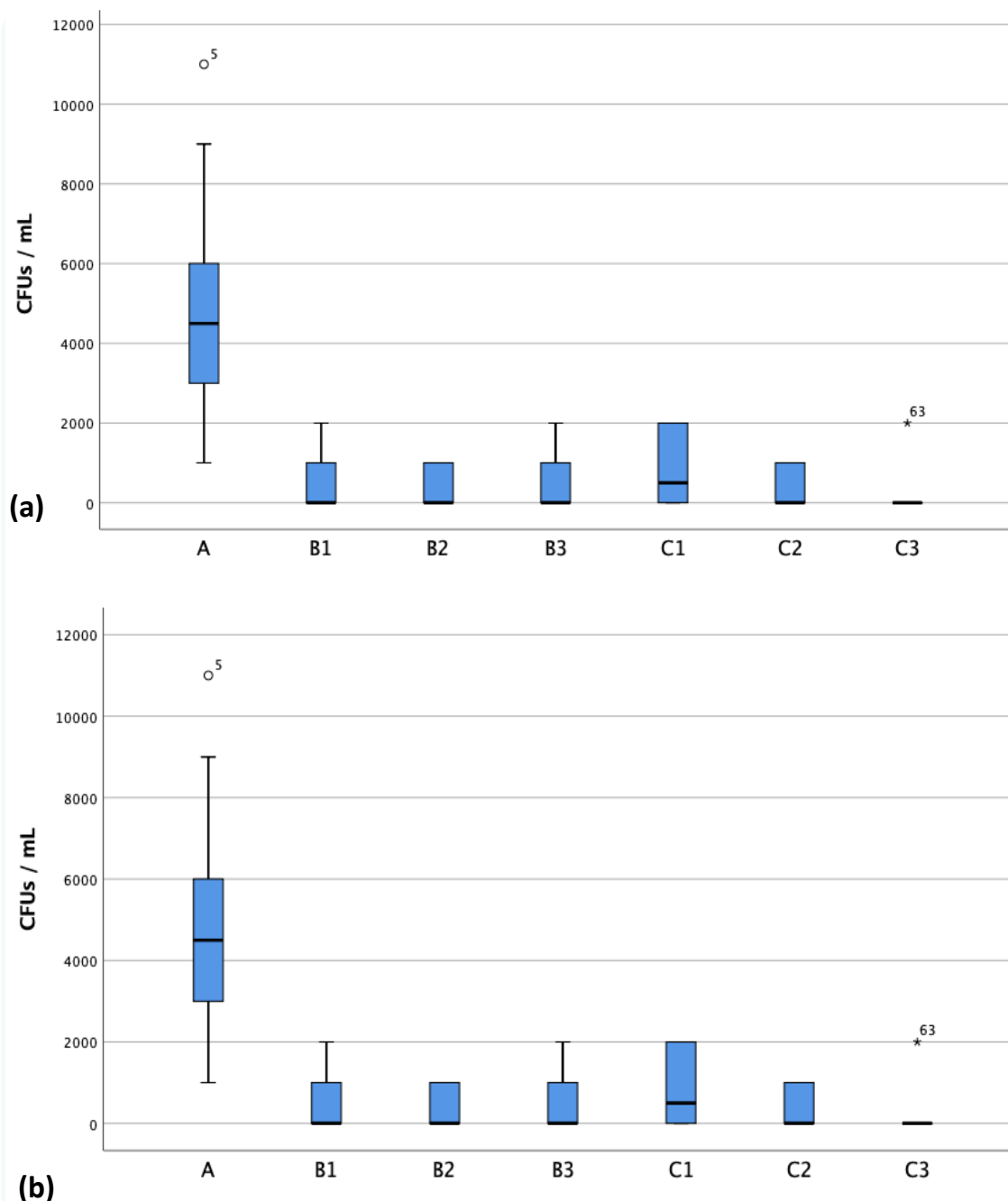


Figure 1: Adhesion assay: CFUs/mL of *candida albicans* formed in unaged (a) and aged (b) specimens of control and experimental groups.

The null hypothesis was rejected since adding DMAHDM in different concentrations (5%, 10%, and 20%) alone or with Nystatin (500000 IU) into heat-cured PMMA resin specimens had increased antimicrobial activity property against *C. albicans* as compared to control samples. The results of CFU count in this study confirmed a significant reduction in fungal attachment on the surfaces of DMAHDM alone and in combination with Nystatin impregnated PMMA samples compared to those of the control group. The current study of antimicrobial activities agrees with the previous research^{18,31} where QAS-based acrylic resin was studied against *C. albicans*. The antimicrobial inhibition was enhanced by increasing the concentration of DMAHDM from 5% to 20%. This might be due to the increase in the charge density of the PMMA resin,

resulting in higher inhibition capacity.³² These charges present in the amine portion of the molecule of DMAHDM promote attraction and interaction with the microbial membrane and its rupture. In addition, charges could damage microbial DNA, disturbing the flow of essential ions and disrupting protein activity.³² The DMAHDM is a quaternary ammonium monomer with an alkyl chain length of 16, which exhibited the greatest antimicrobial potency amongst groups of QAMs tested.²⁰ The longer chain length might inhibit more significant microbial growth.^{18,33} Another reason for the increased activity against *C. albicans* of a higher concentration of DMAHDM might be due to the antimicrobial mechanism of this salt. The positively charged quaternary ammonium adhere to the negatively charged cell membrane in this mechanism. The

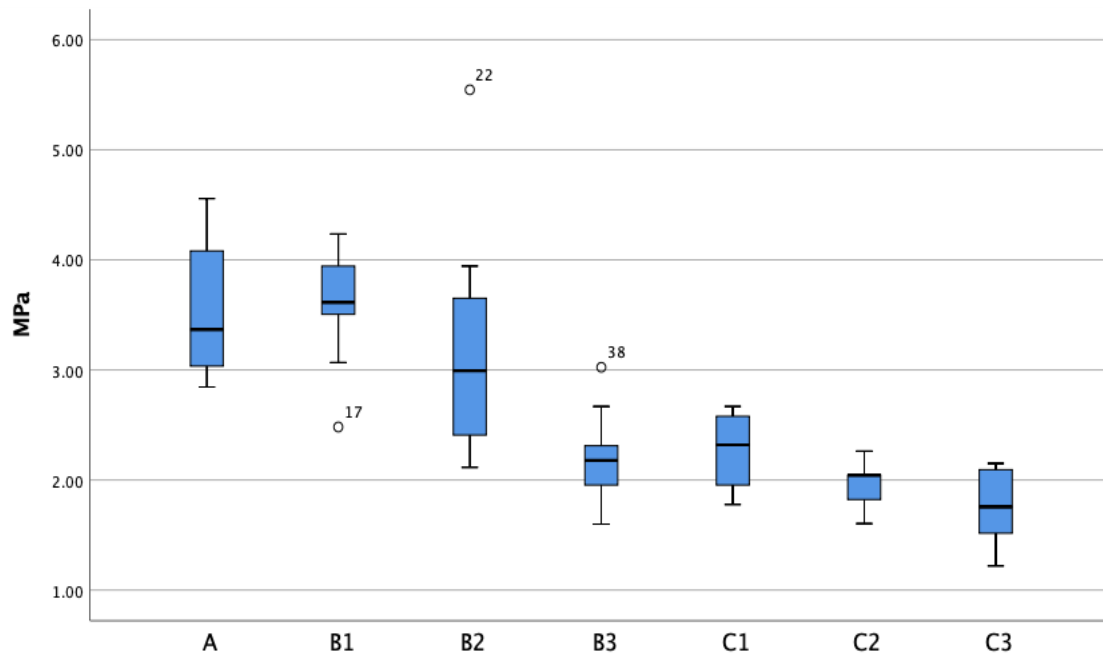


Figure 2: Comparison of Impact strength (MPa) of denture base materials modified with different concentrations of DMAHDM alone and with Nystatin.

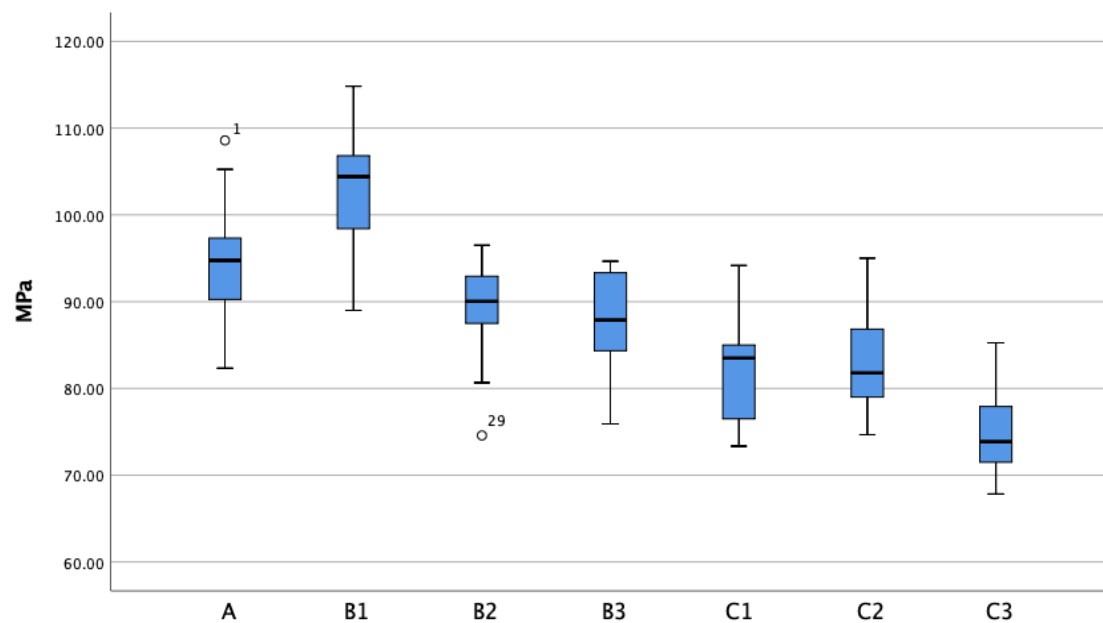


Figure 3: Flexural strength (MPa) of denture base materials modified with different concentrations of DMAHDM alone and with Nystatin.

divalent cations Ca²⁺ and/or Mg²⁺ that cross-bridge the outer membrane is replaced by the charged amino groups of QAM salt. An electrical imbalance might disrupt the cell wall, resulting in cytoplasmic leakage diminishing the survival of fungus and ultimately microbial death.²⁴ The phenomenon of antimicrobial action of the QAS has been described as the “contact killing” mechanism.³⁴

The combination of two antimicrobial agents of DMAHDM (5%, 10%, and 20%) and Nystatin in heat-cured PMMA resin exhibited and maintained strong synergistic effects toward *C. albicans*. Similar antimicrobial results were found in other studies where two different antimicrobial agents have been incorporated in

restorative materials.³⁵ The synergistic effect of Nystatin and contact active DMAHDM might be attributed to the fact that Nystatin might disrupt the permeability and structures of candidal cell membrane. This might be done by targeting ergosterol, the most efficient sterol in mediating nystatin-induced membrane pore formation, suggesting that sterol specificity underlies Nystatin’s antifungal activity,³⁶ and this might further facilitate the contact killing mechanism of DMAHDM. Nystatin used in this study has been previously incorporated in heat cured PMMA acrylic resin as an antifungal agent.³⁷

This current study also investigated the effect of aging acrylic specimens of control and experimental groups in distilled water for 30 days on microbial activities. The most important aspect of the antimicrobial denture base materials is the stability of the resulting efficacy. Regarding the durability of the antimicrobial ability, QAMs in resins have achieved long-lasting antimicrobial ability because the QAM copolymerizes with the resin by forming a covalent bond with the polymer network. Therefore, the QAM is immobilized in the resin and is not lost over time, thus imparting a durable antimicrobial capability.^{15,38} It is reported that a restorative material containing dimethyl amino dodecyl methacrylate (DMADDM) demonstrated that the antimicrobial properties had not decreased after aging in water.³⁹ This is in line with the current study, which also showed no decrease in antimicrobial activity after aging.

Previous research found that human gingival fibroblasts and odontoblasts are biocompatible with QAS.^{40,41} Keke Zhang *et al.* demonstrated that less than 20% QAS in denture material did not raise the inflammatory response *in vivo*, implying good biocompatibility and biosafety of the newly synthesized material using QAS as an antimicrobial addition.³⁴ Furthermore, it was demonstrated that the antimicrobial-compound QAMs were stable and did not release from the material into the saliva.⁴² Quaternary ammonium monomers (QAMs) can cause stress to the cells they come into contact with and the surrounding environment's outer cells. Microbial lysis on the resin surface by QAM has been demonstrated to behave as a stressful environment, initiating programmed cell death (PCD) in the microorganisms around it.⁴² It has been observed that DMADDM-containing resin can kill the entire biofilm, not only the microbes that come into touch with it.⁴³ Quaternary ammonium monomer was added to glass ionomer cement (GIC) and the release of QAM was determined. According to the findings, the release was not detected in saliva.⁴⁴ Hence, the DMAHDM impregnated PMMA resin is an antifungal and biosafe substance.

Despite improvement in the antimicrobial properties of PMMA resin, the impact on its mechanical properties must also be evaluated. In this *in vitro* study with respect to impact strength, the mean value decreased with increasing concentration of DMAHDDM alone or in combination with Nystatin. However, the differences in the mean values between groups A (control) and B1 (5% DMAHDDM) as well as B2 (10% DMAHDDM) were not statistically significant ($p = 1.000$ and 0.706 respectively). This result was in accordance with the previous investigations.⁴⁵ However, the impact strength decreased significantly with 20% of DMAHDDM alone and with 5%, 10%, and 20% of DMAHDM combined with Nystatin. Therefore, the null hypothesis is partially rejected for the parameters tested. The lower impact strength of the specimens of modified materials might be attributed to the formation of micro-defects due to linear side chains in the PMMA matrix. These micro-defects act as stress concentrators

and crack accelerators.⁴⁶ Despite the progressive reduction in impact resistance with the increase in DMAHDDM or in combination with Nystatin, the measured impact energy values satisfied the requirements of the American Dental Standards Institute no.12.⁴⁶

Flexural strength has been used as one of the parameters for evaluating the mechanical properties of denture base resins in this study because this test closely simulates the type of loading conditions *in vivo*.⁴⁷ The addition of DMAHDM alone to the PMMA resin did not significantly change flexural strength. The incorporation of 5% and 10% DMADDM and DMAHDM did not affect the flexural strength of the resin.

According to ISO 20795-1:201327, the minimum flexural strength of heat-polymerizing acrylic resin should be 65 MPa. In this study, the flexural strength of the tested specimens was more than 65 MPa, after immersion for 48 h. Therefore, the results are in compilation with the requirement of ISO 20795-1:201327 regarding this parameter. The samples fabricated with no addition of QAS and Nystatin (controls) showed the highest flexural strength values, while the addition of 20% QAS and Nystatin showed the lowest values. The observed increase in flexural strength values of group B1 containing 5% DMAHDDM might be attributed to reactive moieties. The acrylic moieties of diacrylates are generally more reactive than the methacrylic moieties of dimethacrylates, thus relied upon when faster reaction kinetics are desired.⁴⁸ The addition of 10% and 20% DMAHDM alone or in combination with Nystatin in the tested resin reduced the flexural strength by 8-20 MPa.

The experimental groups had the most significant amounts of antimicrobial agents added. Since DMAHDM is a polar and hydrophilic molecule⁴⁹ and the polarity of the antimicrobial resin appears to dictate water sorption.⁵⁰ It might be argued that more DMAHDM molecules could increase the hydrophilicity (due to the presence of QAS) of resin.⁵¹ The plasticization of the material may explain the different effects on flexural strength due to the hydrophilic characteristic of QAS.⁵² It could be hypothesized that the QAS incorporation increases the intermolecular distance of monomers, affecting polymer chains and consequently decreasing resistance values as far as the flexural strength is concerned.⁵² It is essential to realize that the conversion degree of PMMA resin relating to the amount of residual monomer may influence the values obtained.⁵³ The decrease in the degree of conversion and increase in the amount of residual monomers amount could cause a reduction in the flexural strength values. Another reason for reduced flexural strength might be due to a cluster of antimicrobial agents, which formed loosely attached clusters that acted as stress concentrating centers in the matrix and adversely affected flexural strength. In addition, there was no chemical bond between thymoquinone (TQ) and PMMA.⁵⁴ Accordingly, Nystatin dispersed in the PMMA matrix might have acted as an impurity, adversely affecting the degree of conversion and leading to an increased level of residual monomer, which acted as a plasticizer and resulted

in weakening of the material.⁵⁵ In addition, the incomplete wetting of Nystatin by the resin might be considered an interfering factor in the integrity of the polymer matrix.

Nevertheless, the addition of small percentages of antimicrobial agents to PMMA may be effective against microorganisms. Therefore, its impact on mechanical properties may be less significant than the potential benefits, especially for patients who do not follow an adequate denture cleaning protocol.⁵³ The considerable advantages of this addition could be for elderly patients with limited manual dexterity or cognitive disturbances.

CONCLUSION

The addition of DMAHDM (5%, 10%, and 20%) alone and with Nystatin to the PMMA denture base resin showed statistically significant antimicrobial activities against *C. albicans* before and after aging for 30 days in water. The addition of 5%, 10%, and 20 % DMAHDM alone did not affect the flexural strength of PMMA resin. However, adding 5%, 10 %, and 20% DMAHDM and Nystatin reduced the flexural strength. The addition of 5% and 10% DMAHDM alone did not affect the impact strength of PMMA resin. However, the addition of 20% DMAHDM alone and 5%, 10%, and 20% DMAHDM and Nystatin had reduced the impact strength of PMMA resin. DMAHDM impregnated PMMA resin is an antifungal and biosafe substance without compromising the mechanical properties. However, more than 10% concentration of DMAHDM can affect the impact strength of denture base resin. More *in vitro* experiments should be done to analyze different types of denture base materials. The assessment of various types of denture base materials based on fabrication techniques or curing procedures may significantly impact microbial development and, as a result, antimicrobial capabilities and mechanical properties.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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